SPERMATOGENESIS OF APHIDS; THE FATE OF THE SMALLER SECONDARY SPERMATOCYTE.

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I. INTRODUCTION.

It has been shown by Morgan and von Baehr that in aphids the primary spermatocyte divides unequally producing larger and smaller secondary spermatocytes. The larger secondary spermatocyte undergoes a second maturation division, and produces two equal-sized spermatids which transform into functional spermatozoa. The smaller secondary spermatocyte, which has received fewer chromosomes is said to degenerate. Only two similar spermatozoa, consequently, are formed from a primary spermatocyte.

Von Baehr (1909 and 1912) states that he very rarely observed the development of the smaller secondary spermatocyte to the prophase of the second maturation division, but that it does not divide. Stevens (1909) says that in a preparation which has unfortunately been lost, the anaphase of the smaller secondary spermatocyte was seen, and that such stage may also be distinguished among the degenerating spermatocytes. Morgan (1915) states: "The small cell is left with two chromosomes and a small amount of cytoplasm. It never divides again, and later degenerates. Stevens was inclined to think that the small cell may

sometimes show a division figure, which subsequently fades away, but I have never seen a case of this kind."

In *Macrosiphum ambrosiæ* and *Neothomasia populicola* I have observed the late telophase of the smaller secondary spermatocytes. In *Stomaphis yanois,* moreover, I have found that the smaller secondary spermatocytes divide and form equal-sized spermatids which are much smaller than the larger spermatids. These smaller spermatids develop and reach the sustentacular cells with the developed larger spermatids; they, however, fail to attach to the sustentacular cells. Thus their development ceases; they, therefore, do not fully transform into spermatozoa, but retrogress and form spherical cells, which attach themselves to the epithelium of the cysts of the testes. A further account of this will appear in the following pages.

The work on the spermatogenesis of *Stomaphis yanois* was done in the Tokyo Higher Normal College, and the work on the other aphids has been done in the University of Chciago. The writer's thanks are due to Prof. F. R. Lillie and Prof. S. Yamanouchi, who gave him many suggestions and much help. The writer also wishes to thank Prof. A. Oka and Prof. U. Takakura for their kindness during his stay in Tokyo. For the identification of the aphids the writer is indebted to Dr. A. L. Quaintance and Dr. A. C. Baker.

II. Methods.

Both the males and parthenogenetic females were fixed in either strong Flemming's, Zenker's or a mixture of absolute alcohol one part, acetic acid one part, and saturated aqueous solution of corrosive sublimate two parts. Sections were cut 3, 5 and 10 micra in thickness; most of them, however, were cut 5 micra thick. They were stained with Heidenhain's iron-hematoxylin followed by eosin or borax carmin.

III. Stomaphis yanois.

1. *Primary Spermatocyte.*

Figs. 1 and 2 show the primary spermatocyte prophase. In Fig. 1, one of the chromosomes is formed, and in Fig. 2, the proc-

ess of the formation of the chromosomes is almost finished. There are ten chromosomes, five larger and five smaller, in the equatorial plate of the first spermatocyte division, and they are connected with one another by linin threads as is shown in Figs. 4 and 5. The side view of the mitotic figure shows centrosomes of about the same size agreeing with von Baehr's observation on *Aphis salicti* (Fig. 3).

In the anaphase unequal cell division is indicated. The larger and smaller daughter cells are connected by a bridge of cytoplasm, and elongated lagging chromosomes lie between the chromosomes passing to the daughter cells (Fig. 7). The lagging chromosomes do not show any tendency to go to the larger cell at this time, but after the nuclear membrane is formed, the lagging chromosomes enter the larger cell (Fig. 8). It is interesting to note that the size of the nuclear membrane is larger in the larger cell. The inequality of the size of the nuclei of the daughter cells, therefore, does not seem to be due to the unequal number of the chromosomes, but to an unequal quantity of cytoplasm. In a case where the two daughter cells were about equal the size of the nuclear membrane was about the same.

I have observed many cases in which the lagging chromosomes appear to be divided, but I doubt that this ever occurs. Morgan (1909) states: "That artificial conditions, such as handling or osmosis, might break such a delicate connection at this time is not at all improbable, and such an artificial result might give the impression that the accessory is actually divided. Moreover, if the bridge arches toward or away from the observer, the effect may be produced at certain focal levels of discontinuity between the ends of the lagging chromosomes, when none such exist."

The larger secondary spermatocyte receives eight divided and two lagging undivided chromosomes, and the smaller secondary spermatocyte receives eight divided chromosomes.

2. *Larger Secondary Spermatocyte.*

The larger secondary spermatocyte undergoes an equal second division without an intervening resting stage. The equatorial plate (Figs. 12 and 13) of the second division shows ten chro-

mosomes. In the first maturation division five of the ten chromosomes are larger and five of them are smaller, but in this case six are larger and four are smaller. The reason for this is discussed later on. As in the case of the first division, chromosomes are connected by linin threads. When the split chromosomes shift to the opposite poles interzonal fibers appear. In the first division the middle part of the two daughter cells becomes narrow and shows an appearance of a dumb-bell with the ends different in size. In this case, however, the middle part is broad, so that the interzonal fibers are separated (Fig. 15).

3. *Smaller Secondary Spermatocyte.*

The smaller secondary spermatocyte shows chromosomes in its nuclear cavity at the telophase of the first maturation division. It is not difficult to distinguish the smaller secondary spermatocyte as their diameter is hardly half that of the larger ones. The nucleus does not enter a resting stage. I have found in some cases two small bodies near the nuclear membrane (Fig. 9). These seem to be centrosomes, but I am unable to speak with certainty. The changes in preparation for the second division are similar to those of the larger spermatocyte.

The equatorial plate (Figs. 20 and 21) shows eight chromosomes as compared with ten chromosomes in the equatorial plate of the larger secondary spermatocyte. The cases which distinctly show eight chromosomes are rare; there can be little doubt, however, that this is the full number since there are ten chromosomes in the equatorial plate of the first maturation division, and two of them pass to the larger one as the lagging chromosomes. Four chromosomes are larger and the other four are smaller. There are five larger and five smaller chromosomes in the equatorial plate of the first division; the lagging chromosomes, therefore, must be a larger and a smaller chromosome. If all the chromosomes were to divide in the first division, five larger and five smaller chromosomes would appear in the equatorial plate of the second division. Two chromosomes, one larger and one smaller, lag and enter the larger cell without dividing. The smaller of the lagging chromosomes, consequently, becomes larger than the

other smaller chromosomes. This must be the reason why we see four smaller chromosomes in the larger secondary spermatocyte instead of five.

The side view of the metaphase of the smaller spermatocyte differs from that of the larger one in shape. It is more spindle-shaped (Fig. 22). Fibers are not seen distinctly in the preparations stained with iron hematoxylin. The two stained bodies on both sides of the chromosomes in the equatorial plate might be the centrosomes (Fig. 23). There are cases which show separated chromosomes, and cases which show massed chromosomes (Figs. 23–25). So far as my observation goes, in most cases the chromosomes seem to fuse soon after their splitting. The telophase does not show distinctly the interzonal fibers as in the case of the larger cell. Two equal smaller spermatids are produced after the division.

4. *Smaller Spermatid.*

The germ cells of each cyst of the testes are generally in about the same stage. When the spermatids are young the cysts are spherical in shape, but they elongate during the development of the spermatids. The young smaller spermatids (Fig. 28) have condensed nuclei, but the larger spermatids (Fig. 18) between which they lie have vesicular nuclei. These smaller and larger spermatids are seen all through the cyst. I have examined many cases in order to see whether the polarities of the larger and smaller spermatids are established with relation to the epithelium or not. Most of the young larger and smaller spermatids, which are seen near the epithelium, develop their tails toward the center of the cyst, but some of them may develop along the epithelium or develop their tails toward the epithelium. Those in the central part do not show any definite orientation, and in extreme cases spermatids existing side by side may show opposite directions. In cysts in which the larger spermatids are developed to the stage shown in Fig. 18, the orientation of the larger and smaller spermatids remains unchanged. In a little later stage, however, all the larger and smaller spermatids begin to orient in the same direction, and when the larger spermatids develop to the stage shown in Fig. 19, all are oriented in the same direction.

There must be an interaction, probably chemical, between the sustentacular cells and the larger and smaller spermatids. The larger and smaller spermatids in the outer part opposite the sustentacular cells and in the central part of the cyst generally move among the tails of the other spermatids toward the sustentacular cells, but those in the other parts move toward sustentacular cells along the epithelium.

Developed smaller spermatids (Fig. 31) are seen among the larger spermatids near the sustentacular cells, and do not show any inferiority to the larger spermatids in moving toward the cells. Before the nucleus of the larger spermatid shows marked differentiation the smaller spermatids have retreated a little towards the interior. In other words, well developed smaller spermatids approach towards the sustentacular cells, but do not attach to them. I have examined many smaller spermatids in order to see whether they develop apical parts. Figure 30 shows a developing smaller spermatid, which has a cone-shaped apical part. There is a developed smaller spermatid, which seems to have a well-developed apical part, but we cannot distinctly observe since it is seen in close contact with the tails of the larger spermatids. In most of the smaller spermatids, which have elongated tails, I have not, however, observed developed apical parts.

As to the interpretation of the cells identified as smaller spermatids, may they not be degenerating larger spermatids? So far as my observation goes the larger spermatids rarely degenerate; moreover, it is not hard to distinguish degenerating young larger spermatids from the smaller spermatids, since the former are not only much larger than the latter, but the nucleus of the larger spermatid becomes vesicular while the nucleus of the smaller spermatid is condensed. If the larger spermatids developed to the stage shown in Fig. 19 begin to degenerate, we can recognize them by the difference in the state of the nuclei. If the almost fully developed spermatids begin to degenerate, it is quite easy to tell them from the smaller spermatids, since they have very slender nuclei and the smaller spermatids, which are seen in the same cyst with them, have spherical nuclei. If degeneration of the larger spermatids should occur at the stage in which they

have condensed ovoid nuclei which elongate later, the criterion by which to distinguish them is their position, since when they have developed to such a stage, the smaller spermatids with condensed spherical nuclei have already left the epithelium.

The metaphase of the smaller secondary spermatocytes are seen among those of the larger secondary ones; I think, therefore, there is no doubt that the smaller secondary spermatocytes undergo the second division. More developed larger spermatids are seen with more developed smaller spermatids in the same cyst. We may conclude from these observations that the smaller spermatids develop with the larger spermatids.

I have observed cases where the larger and smaller spermatids are seen in the central part of the cyst, while the majority of spermatids have already reached the sustentacular cells. Such larger and smaller spermatids might fail to reach the sustentacular cells, since they have to move among the spermatids. The examination of the later stages, however, has shown that they succeed in reaching the sustentacular cells.

Figure 32 shows a smaller spermatid which is abnormally big and has a distinct axial filament. Ordinarily the smaller spermatids elongate similarly, but are more delicate. One of the most developed smaller spermatids is shown in Fig. 33. In such a stage their development comes to a standstill, and they begin to retrogress. They gradually retreat toward the tails of the larger spermatids. Their nuclei which are deeply stained with iron hematoxylin are seen among the tails of the larger spermatids in a somewhat regular position. Finally they pass out to the cavity of the cyst.

The smaller spermatids fail in attaching to the sustentacular cells; they cannot, consequently, get material for their further development. They have to live on their own substance. Their tails become shorter, and the cytoplasm around the nucleus increases (Fig. 34).

The forms shown in Fig. 35 are seen near the tail of the fully developed functional spermatozoa in the cavity of the cyst. We do not see such spermatids in the cavities of the cysts at the younger stages. These smaller spermatids still have elongated tails, but later transform into spherical cells which have a distinct cell

membrane (Fig. 39) and show a tendency to fuse with each other. There are some cells which have two or more condensed nuclei. These seem to be the products of the fusion of two or more smaller spermatids. Some of the retrogressed cells of the smaller spermatids attach to the epithelium, and on these cells other cells attach themselves; thus they form layers as shown in Fig. 38. In other cases they are irregularly attached to the epithelium. When they attach themselves to each other they show a polygonal shape.

A, b and *c* in Fig. 38 are parts of adjacent cysts, where fully developed spermatozoa occur though not shown in the figure. The cells occurring between the cysts are the retrogressed smaller spermatids produced in the cyst *c,* and the epithelium proper is very thin as seen between cysts *a* and *b*. As we see in the figure these cells are not equal in size. In some of them the nuclei are broken up and their fragments are seen scattered throughout the cells. The others still show condensed spherical nuclei. As stated already the larger spermatids rarely degenerate. These larger spermatids may become like the cells just mentioned. Though degenerating larger spermatids mingle among these cells, there is no criterion by which they may be distinguished from retrogressed cells of the smaller spermatids.

Some of these cells may be absorbed by the epithelial cells, but how far the absorption proceeds is at present undetermined. When these cells attach to the epithelium the functional spermatozoa are already fully developed. Afterwards the wall of the cyst ruptures, and these cells being deprived of their connection with the testis are destined to disappear. It is possible that they are extruded from the testis along with the spermatozoa. I have observed epithelial cells of the cyst and retrogressed cells of the smaller spermatids in some of the vasa deferentia. The sections of the testes of the old males show remarkable changes. Their walls are thickened and neither spermatozoa nor the cysts, which fill the young testes, can be seen.

IV. Neothomasia populicola and Macrosiphum ambrosiæ.

The testes of embryos of *Neothomasia populicola* and *Macrosiphum ambrosia* are in the early stages, but those of larvæ are

suitable for the purpose of studying the spermatocyte divisions. As is the case in other aphids, the primary spermatocytes of these aphids divide unequally, and the anaphase shows the lagging chromosomes. I have found in these aphids telophases of the second maturation division of the smaller secondary spermatocyte, but have observed no developing smaller spermatid; we may, therefore, conclude that the smaller secondary spermatocytes and the smaller spermatids of these aphids degenerate as in the cases of the aphids studied by Morgan, von Baehr and Stevens. In *Macrosiphum ambrosia* I observed cases in which all smaller secondary spermatocytes seemed to be dividing, but I will conclude in a succeeding paper whether all the smaller secondary spermatocytes divide or not.

In the cyst, where larger spermatids are already attached to the sustentacular cells, there are seen spermatids which look like the smaller spermatids of *Stomaphis yanois*. As stated above, since no development of the smaller spermatids was observed, they must be larger spermatids. In slightly younger cysts some spermatids are seen among the developed tails of other larger spermatids, which are about to attach to the sustentacular cells. Such spermatids probably have no chance of reaching the cells. I have found cases in which the larger spermatids are already attached to the cells, but some spermatids are seen among the ends of the tails of the larger spermatids. In other cysts spermatids with condensed nuclei are seen apart from the sustentacular cells, while others are attached to them.

As in the case of *Stomaphis yanois* young spermatids of these aphids change their orientation to the same direction; some spermatids, therefore, move to the sustentacular cells across the whole diameter of the cyst or reach the cells moving along the epithelium. If they move to the sustentacular cells along the epithelium, as most of the spermatids do, they may lose the chance to become attached to them. Developed spermatids have been found by the side of spermatids which are attached to the sustentacular cells and are developing. They were probably prevented from reaching the cells by other spermatids, and their development came to a standstill; they, consequently, show younger stages than the spermatids which are attached to the

cells. Since many spermatids are produced in the cysts, if they move to the sustentacular cells through the tails of other spermatids, they meet much resistance; they, therefore, might be unable to reach the cells.

The most conspicuous difference between the case of *Stomaphis yanois* and that of these aphids is the position of the retrogressing spermatids. In the former case the smaller spermatids approach the sustentacular cells, and then gradually retreat toward the tails of the larger spermatids; their position, consequently, is regular, having a relation to the development of the larger spermatids. In the latter case, however, the position of the retrogressing spermatids is irregular.

As in the case of *Stomaphis yanois* retrogressed spherical cells are seen in the cyst with fully developed spermatozoa. These cells attach themselves to the epithelium of the cysts and have the same fate as the retrogressed cells of the smaller spermatids of *Stomaphis yanois*.

V. Review.

According to Meves and others, one of the secondary spermatocytes of the honey bee is much smaller than the other, and receives no chromosomes; it, consequently, degenerates after some time. The larger secondary spermatocyte, moreover, divides unequally in the second spermatocyte division. The chromosomes divide this time, and there are produced larger and smaller spermatids. The larger spermatids differentiate into functional spermatozoa. The smaller spermatids also undergo some differentiation which, however, comes to a standstill at a late stage and then they degenerate without transforming into functional spermatozoa. The smaller spermatid of *Stomaphis yanois* resembles that of the honey bee in some respects. Both of them are much smaller than the larger spermatids, but judging from the Meves' drawings, the difference in size between the larger and the smaller spermatids is greater in the honey bee than in the aphid. They both develop to some extent, but do not transform into functional spermatozoa. Meves does not state what kind of changes occurs in the degenerating smaller spermatids of the honey bee; I am, therefore, unable to compare their later stages with those of the smaller spermatids of *Stomaphis yanois*.

The most conspicuous difference between the smaller spermatids of the honey bee and this aphid is seen in the nuclei. The nucleus of the smaller spermatid of the honey bee returns to a resting stage, and differentiates similar to that of the larger spermatid. The nucleus of the smaller spermatid of this aphid, however, becomes condensed after the second spermatocyte division, and remains in the same state, although the cytoplasm shows changes similar to those of the larger spermatid. This may be caused by the absence of the lagging chromosomes in the smaller spermatids, while in the honey bee the smaller spermatids have the same number of chromosomes as the larger spermatids.

Whitney (1918) mentions that the normal and rudimentary spermatozoa have been found in considerable number of rotifers. In his paper of 1917 he says that the functional spermatozoa are identical in their power of determining the sex of the individual that develops from a fertilized egg, since after a functional spermatozoon has fertilized a parthenogenic male egg, the egg always develops into a female individual.

In the case of these rotifers, according to Whitney, the chromosomes divide in the first spermatocyte division. One half of the secondary spermatocytes divide and form the normal spermatids. The remaining half of the secondary spermatocytes, contrary to the case of the smaller secondary spermatocyte of *Stomaphis yanois,* do not divide, but develop directly into the degenerate spermatozoa. The spermatocytes destined to degenerate are smaller than the others, and their development into the complete rudimentary spermatozoa is strikingly different from the development of the normal spermatids.

Whitney ('18) says that as all the fertilized eggs in both phylloxerans and rotifers develop into female young, it seems safe to conclude, as Morgan has already concluded, that the degenerate sperm cells are the male-determining ones and that the normal sperm cells are the female-determining ones.

Stevens (1905) found many degenerate spermatozoa in *Blattella germanica.* She states that the distribution and varying number of these degenerate spermatozoa make it impossible to interpret their condition as due to the absence of the accessory chromosome as Miss Wallace does in the spider, and that the only

probable explanation seems to lie in the imperfect mitosis. She detected no evidence of degeneracy among the young spermatids.

VI. Summary.

1. In *Stomaphis yanois* the smaller secondary spermatocytes divide, and develop to some extent, but retrogress to spherical cells.

2. In *Neothomasia populicola* and *Macrosiphum ambrosia*, cases of division of the smaller secondary spermatocytes were found, but no developing smaller spermatids were observed.

3. In *Neothomasia populicola* and *Macrosiphum ambrosia* spherical cells like those in *Stomaphis yanois* were found in the cysts containing spermatozoa. These were identified as retrogressed larger spermatids.

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EXPLANATION OF PLATES.

All of the drawings were made with the aid of camera lucida. Figs. 1 to 15 were drawn with a Leitz 1/16 oil immersion objective and a Zeiss compensating ocular 18. Figs. 16 to 37, except Fig. 33, were drawn with a Leitz 1/16 oil immersion objective and a Leitz ocular 5. Fig. 33 was drawn with a Leitz 1/16 oil immersion and a Leitz ocular 4. Fig. 38 was drawn with a Leitz 1/16 oil immersion objective and a Leitz ocular 3. All figures from *Stomaphis yanois*.

PLATE I.

FIGS. 1 AND 2. Primary spermatocytes, prophase.
FIGS. 3, 4, 5 AND 6. Primary spermatocytes, metaphase.
FIG. 7. Primary spermatocyte, anaphase.
FIGS. 8 AND 9. Primary spermatocytes, telophase.

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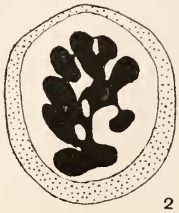

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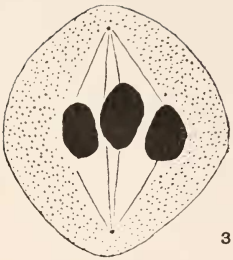

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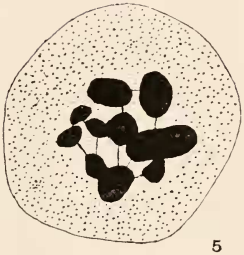

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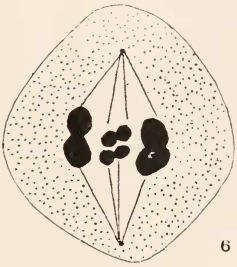

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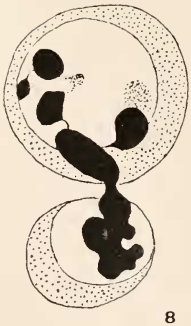

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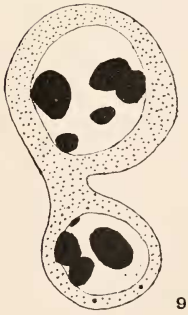

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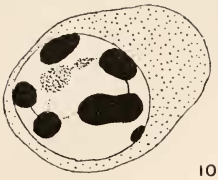
Plate II.

Fig. 10. Larger secondary spermatocytes, prophase.
Figs. 11, 12 and 13. Larger secondary spermatocyte, metaphase.
Figs. 14 and 15. Larger secondary spermatocytes, anaphase.
Fig. 16. Larger secondary spermatocyte, telophase.
Figs. 17, 18 and 19. Larger spermatids.



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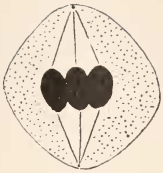

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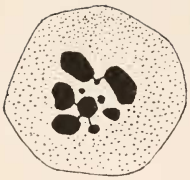

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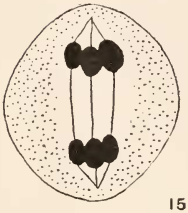

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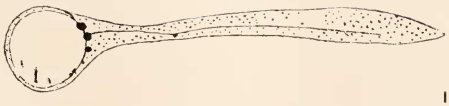

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Plate III.

Figs. 20, 21, 22, 23 and 24. Smaller secondary spermatocytes, metaphase.
Figs. 25 and 26. Smaller secondary spermatocytes, anaphase.
Fig. 27. Smaller secondary spermatocyte, telophase.
Figs. 28, 29, 30 and 31. Smaller spermatids.

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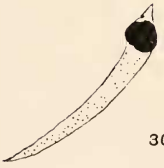

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Plate IV.

Figs. 32 and 33. Smaller spermatids.
Figs. 34, 35 and 36. Retrogressing smaller spermatids.
Fig. 37. Retrogressed cell of the smaller spermatid.
Fig. 38. Layers of the retrogressed cells.

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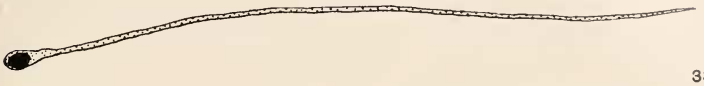

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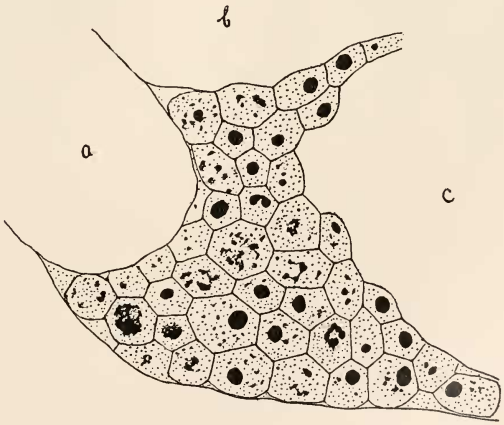

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